

Multiple Mating of Male and Female Codling Moth (Lepidoptera: Tortricidae) in Apple Orchards Treated with Sex Pheromone

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ABSTRACT Studies were conducted with codling moth, *Cydia pomonella* L., to evaluate the mating status of male and female moths in apple, *Malus domestica* (Borkhausen), orchards treated with and without sex pheromone dispensers. Laboratory studies first examined the effect of multiple mating of male and female moths on female fecundity and egg fertility. Females that had mated three times had a significantly higher fecundity than singly mated moths. Sequential mating by male moths had no effect on the fecundity of female moths or egg fertility. However, male moth age did impact female fecundity, with significantly fewer eggs laid after mating with virgin 1- versus 3-d-old males. The mean size of the first spermatophore transferred by males was significantly larger than all subsequent spermatophores. Classifying spermatophores based on size was used in field sampling to categorize the mating status of the female's partner. The proportion of mated females with small spermatophores (partner had previously mated) was significantly higher in treated versus untreated orchards. The proportion of female moths caught in traps baited with pear ester that were virgin was low (≤ 0.26) in both treated and untreated orchards. The proportion of females with more than one spermatophore was low (≤ 0.06) in treated orchards all season and during first moth flight in untreated orchards (0.11). Nearly one third of female moths, however, had more than a single spermatophore in untreated orchards during the second moth flight. The potential impacts of multiple mating and delayed mating by male and female codling moth on the effectiveness of sex pheromones are discussed.

KEY WORDS apple, *Cydia pomonella*, behavior, traps, monitoring

Various formulations of the sex pheromone of codling moth, *Cydia pomonella* L., have been developed and implemented for mating disruption in apple, pear, and walnut orchards to manage this key pest (chopped fibers: Moffitt and Westgard 1984; aerosol puffers: Shorey and Gerber 1996; hand applied dispensers: Thomson et al. 2001; microencapsulated sprayables: Knight and Larsen 2004). Adoption of sex pheromones has been credited with allowing growers to significantly reduce their use of organophosphate insecticides (Calkins 1998). However, for many other growers, the use of sex pheromones has not been a panacea, and most orchards in the western United States continue to be treated with supplemental insecticide sprays for codling moth (Alway 1997).

Monitoring female codling moths with traps baited with ethyl (*E*, *Z*)-2,4-decadienoate (pear ester) has revealed that a large proportion of female moths are mated in sex pheromone-treated orchards (Light et al. 2001, Knight and Light 2005, Light and Knight 2006). These findings suggest that reductions in fruit damage by codling moth observed in orchards treated with sex pheromones may be caused by a combination of mat-

ing disruption and a delay in mating (Knight 1997). Laboratory studies have shown that female moths that mate at an older age have significantly lower fecundity and egg fertility (Knight 1997, Vickers 1997).

Female codling moths can mate during the first scotophase after eclosion (Howell 1991). Multiply mated female codling moths generally constitute 10–40% of field populations in orchards not treated with sex pheromone (Gehring and Madsen 1963, Hagley 1974, Howell 1988, Light and Knight 2006). The occurrence of multiply mated females in sex pheromone-treated orchards has recently been briefly reported (Light and Knight 2006). However, whether multiple mating of female codling moth in the field affects either fecundity or egg fertility is unknown.

Moth age and sexual experience can also have significant effects on the subsequent reproductive fitness of male codling moth. Males can mate at the start of the first scotophase after emergence (Howell et al. 1978); mating success peaks with 3-d-old males under optimal laboratory conditions (Deseö 1971). Males under laboratory conditions mate on average three to four times (Howell 1991). Spermatophore size is reduced after the first mating (Howell et al. 1978). For example, >90% of male's first spermatophores were >1.5

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mm, whereas all subsequent spermatophores passed by males were ≤ 1.5 mm. Spermatophore size does not seem to affect female's fecundity (Deseö 1971), but the number of previous mating events by males significantly impacted female fecundity (Howell 1980a), egg fertility (Deseö 1971), or both (Hagley 1974) in tests with different laboratory strains. Unfortunately, the interaction of male moth age and mating order has not been examined in these studies. Vickers (1997), however, found with naïve male moths no effect of age (2 versus 6 d) on female fecundity or egg fertility.

Nearly 40% of female moths caught in light traps placed in abandoned apple orchards had small spermatophores (≤ 1 mm) (Howell 1991). Several factors can contribute to the transfer of a small spermatophore including mating order and the interruption of mating (Howell et al. 1978). Howell (1980b) noted that a shift in the mean size of spermatophores occurred in orchards where mass-trapping of males with sex pheromone-baited traps was used. This was purported to be because of a decreased male:female sex ratio. Whether the application of sex pheromones for mating disruption of codling moth impacts mate competition among males has not been examined.

Herein, studies examine the effect of multiple mating of female and male codling moths on female fecundity and egg fertility under laboratory conditions. Data on the mating status and spermatophore number and size were also collected from field populations of female codling moth in orchards treated with and without sex pheromone dispensers from 2000 to 2003. The potential effects of multiple mating of male and female codling moth on the effectiveness of sex pheromone dispensers in managing codling moth populations are discussed.

Materials and Methods

General Protocol. All laboratory studies were conducted with a colony of codling moth reared on an artificial wheat germ-based diet (Toba and Howell 1991). Mature larvae inside corrugated cardboard were placed individually in vials and reared at 25°C until adult eclosion. Moths were sexed, stored at 5°C and used in experiments within 24–48 h. All laboratory studies were conducted in rooms maintained at 25°C, >40% RH, and a reversed 17:7-h L:D light regimen. Scotophase started at 1030 hours and ended at 1730 hours. Light levels were maintained at 360 lux during photophase. A simulated 1-h twilight period beginning at 0930 hours was controlled by a series of time clocks that switched off incandescent light sources at 20-min steps to light levels of 114, 18, and 7 lux. These steps were reversed during a simulated sunrise period. Laboratory mating studies were conducted with adults placed in clear 425-ml plastic cups with lids. Moths were moved in and out of cups 1–2 h before the start of scotophase. On test completion, the mating status of females was determined by dissection of the bursa copulatrix using a microscope. Plastic cups were cut into two sections, and the total number of eggs in the cup and on the lid was counted using a

microscope. Eggs were categorized as fertile (white, red-ring, black-headed, and hatched) or infertile. Infertile eggs were characterized as being mostly clear with only a small amount of disorganized embryonic material.

Multiple Mating of Female Moths. A single <24-h-old virgin female moth was placed in plastic cups with three <2-d-old virgin male moths. Moths were removed after 7 d, and females were dissected to determine the number of spermatophores. The number of fertile and infertile eggs in each cup were counted after 14 d. Two hundred sixty-five cups were set up in this test.

Multiple Mating of Male Moths. Male moths <24 h old were placed initially in cups with a 2-d-old virgin female moth. Each female moth was replaced with another 2-d-old virgin female every 24 h for 10 d. Replaced females were transferred to a clean plastic cup. Females were dissected after 7 d, and the number of fertile and infertile eggs laid in cups were counted after 14 d. One hundred ten males were used in this test.

Mating Status of Field Populations. Female codling moths were sampled in commercial apple orchards situated near Brewster, WA (48° N, 119° W) from 2000 to 2002, and near Wapato, WA (46°45' N, 120°42' W) in 2000. Experimental, unsprayed apple orchards were monitored near Yakima, WA (46°33' N, 120°23' W) from 2000 to 2003. All orchards except four experimental blocks were treated with Isomate-C Plus dispensers (Pacific Biocontrol, Vancouver, WA) loaded as per label with 182.3 mg of a 60:33:7 blend of (*E*, *E*)-8-10-dodecadien-1-ol (codlemone), dodecanol, and tetradecanol. The density of dispensers varied among orchards and regions and ranged from 500 to 1,000 dispensers/ha.

Codling moth was monitored in each orchard with a pair of delta-shaped traps baited with a pear ester lure (Pherocon CM-DA; Trécé, Adair, OK). The two traps were separated by 100 m and placed 20–30 m from the physical border in each orchard. Traps were placed in Brewster orchards the first week of May and in the Yakima and Wapato orchards near mid-April. Traps were checked weekly until early September. Lures were replaced every 8 wk, and sticky trap inserts were replaced either weekly or up to a 4 wk interval depending on their condition. The start date for the second moth flight was established as 1 July. Moths were sexed and female moths were dissected in the laboratory to determine their mating status and the number of spermatophores. The maximum width of spermatophores was measured for all females trapped in eight Yakima orchards during 2003. Four orchards were treated with 1,000 Isomate-C Plus dispensers and four were untreated.

Orchards in Brewster were 8–16 ha and included 'Delicious', 'Golden Delicious', 'Gala', and 'Fuji'. Orchards were planted at 500–1,000 trees/ha, and mean tree heights ranged from 3.0 to 5.1 m. Orchards typically contained a mixture of cultivars with the dominant cultivar comprising >75% of the area. Studies were conducted in 15, 34, and 29 apple orchards dur-

Table 1. Occurrence and influence of single or multiple mating by female codling moth on their fecundity and egg fertility in laboratory studies when a single virgin female moth was placed in a cup with three virgin male moths

No. spermatophores	No. moths	Mean (SE) no. eggs laid	Mean (SE) proportion of fertile eggs
1	89	74.4 (5.9)b	0.83 (0.3)
2	61	90.1 (8.1)ab	0.86 (0.3)
3	31	108.7 (14.2)a	0.85 (0.5)
4	22	74.6 (11.7)b	0.79 (0.5)
ANOVA:		$F_{3,199} = 2.73; P < 0.05$	$F_{3,194} = 0.70; P = 0.97$

Column means followed by a different letter are significantly different (LSD test, $P < 0.05$).

ing 2000, 2001, and 2002, respectively. Orchards were treated with 500–750 dispensers/ha. Orchards in the Yakima and Wapato regions were mixed plantings of ‘Delicious’ and ‘Golden Delicious’ and planted at densities of 500–600 trees/ha. Tree heights averaged 4.0–4.5 m.

Orchards were categorized based on their density of Isomate-C Plus dispensers; having 0, 500–750, and 1,000 dispensers/ha. Moth catch and the proportion of multiply mated female moths in orchards treated with 1,000 dispensers/ha were subdivided into two groups based on large differences in moth population density: 11 commercial apple orchards with moderate moth densities situated near Wapato and monitored in 2000 and 3 experimental orchards with high moth densities situated near Yakima and monitored from 2000 to 2003.

Statistical Analysis. Before analysis, the assumption of normality was tested for all data sets with the Shapiro–Wilk test (Analytical Software 2003). If the null hypothesis that data were normally distributed was rejected, count data were transformed with a square-root transformation and proportional data with the angular transformation to stabilize variances (Snedecor and Cochran 1967). Analysis of variance (ANOVA) was used if the transformed data were normally distributed (Shapiro–Wilk test). Otherwise, the data were analyzed with a Kruskal–Wallis parametric ANOVA of mean ranks (Analytical Software 2003). The relations of male moth age and mating order on spermatophore size, female fecundity, and egg fertility were analyzed independently because of an unbalanced design. The influences of male moth age were also analyzed separately for each male mating order. Linear regression was used to examine the proportion of males successfully mating based on the number of previous successful mating events and moth age. Spermatophore size in females with one or more than one spermatophore was compared with a *t*-test. The proportion of spermatophores that were ≤ 1.5 mm were compared for treated and untreated orchards for females that were mated once or more than once using a χ^2 2 by 2 contingency test. Fisher least significant difference (LSD) test, $P < 0.05$, was used to separate means following significant ANOVAs (Analytical Software 2003). Data for both laboratory females with five to seven spermatophores and males that transferred five to seven spermatophores were not included in the respective ANOVA, because of their small sample size ($< 6\%$ of either data set).

Results

Multiple Mating of Female Moths. More than 80% of females placed with three male moths successfully mated in the plastic cup assay (Table 1). The mean (SE) number of spermatophores was 2.17 (0.09), with a range of 1–7 per female. Spermatophore number had a significant effect on female fecundity (Table 1). Moths with three spermatophores laid significantly more eggs than either females with one or four spermatophores. Female moths with two spermatophores had an intermediate level of fecundity. The influence of spermatophore number on egg fertility was not significant.

Multiple Mating of Male Moths. Greater than 80% of male moths mated with at least one female based on the deposition of a spermatophore (90 of 110 moths tested). The mean (SE) number of females mated by a single male was 1.98 (0.09) with a range of 1–7. The number of consecutive matings by male codling moth had a significant effect on the mean width of spermatophores (Table 2). The first spermatophore deposited was significantly larger than all subsequent spermatophores. The second to fourth spermatophore deposited did not differ in size. Less than 10% of the first spermatophore transferred by males were ≤ 1.5 mm. In comparison, only 6% of the second to fourth spermatophores transferred were > 1.5 mm. Mating order did not significantly affect either the mean fecundity of female moths or egg fertility (Table 2). There was a significant declining linear relationship between the proportion of males (*y*) successfully transferring a spermatophore and mating order (*x*): $y = 0.74 - 0.12x$; $F_{1,5} = 28.14$, $P < 0.01$. The correlation between spermatophore size and female fecundity was not significant ($R^2 = 0.001$, $P = 0.82$).

Male age without regard to the number of previous mating events was a significant factor influencing spermatophore size, female fecundity, and egg fertility (Table 2). Mean spermatophore size declined with increases in male age from 1 to 5 d. There was a significant declining linear relationship between the proportion of males (*y*) successfully transferring a spermatophore and moth age (*x*): $y = 0.64 - 0.16x$; $F_{1,2} = 25.30$, $P < 0.05$. Mean female fecundity was significantly higher when mating with 3-d-old males versus 1-, 2-, 5-, and 7-d-old males, and lower with a 1-d-old versus 2-, 3-, 4-, and 6-d-old males. The proportion of fertile eggs was higher when females mated with 2-d-old versus either 1- or 7-d-old males.

Table 2. Influences of consecutive male mating events and male moth age on spermatophore size, female fecundity, and egg fertility when males were placed in cups with a new, virgin female moth every 24 h

Data	No. moths	Mean (SE) width (mm) of spermatophore [range]	Mean (SE) no. eggs deposited per female moth	Mean (SE) proportion of fertile eggs
Order of consecutive male mating events				
First	87	1.82 (0.02) a [1.4–2.1]	74.2 (5.2)	0.74 (0.03)
Second	51	1.30 (0.03) b [0.8–1.7]	80.1 (5.4)	0.76 (0.04)
Third	28	1.13 (0.03) b [0.9–1.5]	90.7 (7.5)	0.69 (0.04)
Fourth	16	1.03 (0.04) b [0.8–1.5]	73.8 (9.5)	0.65 (0.08)
ANOVA:		$F_{3,178} = 196.0 P < 0.0001$	$F_{3,178} = 1.30 P = 0.28$	$F_{3,172} = 0.75 P = 0.52$
Moth age (d) when mating				
1	47	1.83 (0.02) a [1.5–2.0]	59.1 (6.3) c	0.70 (0.05) b
2	43	1.59 (0.05) b [0.8–2.1]	76.7 (5.1) b	0.84 (0.03) a
3	36	1.45 (0.05) c [0.9–2.0]	102.2 (7.5) a	0.77 (0.04) ab
4	24	1.28 (0.07) d [0.9–2.0]	87.5 (9.3) ab	0.72 (0.04) ab
5	17	1.08 (0.04) e [0.8–1.5]	72.8 (9.6) bc	0.71 (0.07) ab
6	12	1.11 (0.06) de [0.8–1.4]	84.9 (10.2) ab	0.71 (0.07) ab
7	6	1.12 (0.09) de [0.9–1.5]	59.7 (20.7) bc	0.58 (0.15) b
ANOVA:		$F_{6,178} = 32.20 P < 0.0001$	$F_{6,178} = 4.22 P < 0.001$	$F_{6,172} = 2.32 P < 0.05$

Column means with mating order and moth age followed by a different letter are significantly different (LSD test, $P < 0.05$).

Mating Status of Field Populations. The mean catch of female codling moths per trap varied during both the first and second moth flight among the four region–treatment classes (Table 3). Moth catches were significantly higher (four to seven times) in the Yakima experimental orchards than in the commercial orchards monitored in Brewster or Wapato. Mean moth catches were similar between the treated and untreated Yakima orchards and between Brewster and Wapato orchards during both flight periods, respectively.

The proportion of female moths that were mated was high in all orchard groups (>0.70) and did not differ significantly among the groups during either flight period. The proportion of female moths with at least two spermatophores differed significantly among groups of orchards (Table 3). The proportion of multiply mated females was generally lower during the first versus second moth flight. The proportion of female moths with at least two spermatophores was significantly higher in the untreated Yakima experimental orchards compared with female moths sampled in all groups of pheromone-treated orchards. Nearly one third of mated female moths in untreated orchards during the second flight contained at least two spermatophores (Table 3).

Orchards grouped as having either a low or high density of sex pheromone dispensers or untreated also differed in the mean number of spermatophores in females during both flights (Table 4). The mean number of spermatophores was highest in untreated versus treated orchards during both moth flights. A similar pattern was also seen during the second moth flight; however, female mating in orchards treated with only 500–750 dispensers/ha had significantly more spermatophores than in orchards treated with 1,000 dispensers/ha.

Mean spermatophore size in females with only one spermatophore differed significantly in orchards treated with and without sex pheromone dispensers during both flight periods (Table 5). However, mean spermatophore size did not vary based on treatment

with females having more than one spermatophore. During the first moth flight, the mean spermatophore size was larger in female moths with one versus more than one, but was not different during the second moth flight period.

The proportion of females with small spermatophores (≤ 1.5 mm) was significantly higher in treated versus untreated orchards during both flight periods (Table 5). Three of four mated females in treated orchards during the second moth flight had small spermatophores. The proportion of females with small spermatophores was marginally significant ($P = 0.05$) between treatments during first moth flight in females with more than one spermatophore.

Discussion

The effectiveness of sex pheromones to manage codling moth populations is based on the principle of mating disruption (Vickers and Rothschild 1991). However, the need for a broader definition that includes both the prevention and a temporal delay in the mating of moths (Barclay and Judd 1995) is supported by three factors: a majority of female codling moths are mated in treated orchards (Howell and Britt 1994, Knight 2000, Light et al. 2001, Knight and Light 2005, Knight 2006); a delay in mating was shown in sex pheromone-treated versus untreated orchards after field releases of virgin codling moths (Knight 1997); and both fecundity and egg fertility decline with female moth age at mating (Knight 1997, Vickers 1997). Data presented here suggest that the occurrence and prevention of multiple mating by male and female moths, respectively, should also be included in a new, broader definition.

Polyandry has been associated with increased fecundity in numerous lepidopteran species (Torres-Villa et al. 2004), but not previously for codling moth (Howell 1991). Nutritional benefits acquired from multiple mating are thought to benefit species with limited adult feeding (Delisle and Hardy 1997). The importance of adult feeding for codling moth has been

Table 3. Mating status of female codling moths caught on pear ester-baited traps placed in orchards treated with and without Isomate-CM Plus dispensers for mating disruption (MD), central Washington, 2000–2003

Region ^a and years	Treatment ^b	First moth flight			Second moth flight		
		Mean (SE) no. females per trap	Proportion of female moths		Mean (SE) no. females per trap	Proportion of female moths	
			Mated	Multiply mated ^c		Mated	Multiply mated ^c
Brewster 78: 2000–2002	MD _{500–750}	9.2 (1.7) b	0.84 (0.04)	0.02 (0.01) b	5.0 (0.7) b	0.85 (0.02)	0.05 (0.01) b
Wapato 11: 2000	MD _{1,000}	7.9 (1.9) b	0.77 (0.06)	0.01 (0.01) b	11.0 (3.5) b	0.85 (0.04)	0.05 (0.03) b
Yakima 12: 2000–2003	MD _{1,000}	53.1 (11.6) a	0.74 (0.04)	0.01 (0.01) b	44.3 (10.6) a	0.81 (0.02)	0.06 (0.01) b
Yakima 12: 2000–2003	No MD	69.5 (11.9) a	0.87 (0.03)	0.05 (0.01) a	61.8 (13.8) a	0.90 (0.02)	0.32 (0.02) a
ANOVA: df = 3,109		$F = 36.38 \ P < 0.0001$	$F = 1.09 \ P = 0.36$	$F = 5.96; \ P < 0.001$	$F = 47.38 \ P < 0.0001$	$F = 1.09 \ P = 0.36$	$F = 23.44 \ P < 0.0001$

Column means followed by a different letter are significantly different (LSD test, $P < 0.05$).

^a The number represents the no. of orchards monitored.

^b Orchards were treated with sex pheromone for mating disruption (MD) of codling moth. The subscript represents the numbers of Isomate-C Plus dispensers applied per hectare in these orchards.

^c Females with at least two spermatophores.

variable in laboratory studies (Howell 1991), and has not been examined in the field. Codling moth is polyandrous under crowded laboratory conditions (Howell et al. 1978) and in orchards with high population densities (Howell 1988), but the occurrence of polyandry in low-density codling moth populations (found in most commercial orchards) has not been examined. Interestingly, polyandry was rare in orchards treated with sex pheromones, regardless of moth density.

The potential impact of reducing female multiple mating in sex pheromone-treated orchards can be estimated and shown using data with laboratory moths (Table 1) and the density distribution of spermatophore number in populations from untreated and sex pheromone-treated orchards (Table 4). Based on these data, female fecundity would have been 7% lower in orchards treated with 1,000 dispensers/ha compared with untreated orchards. The combined reductions in female fecundity in orchards treated with sex pheromone dispensers from reduced mating (8%), reductions in multiple mating (7%), and a delay in mating (40%) together could have a significant effect on the net reproductive potential of codling moth. However, especially in orchards with moderate population densities of codling moth, these factors may not be sufficient to adequately manage this direct pest because of its low economic threshold (Wearing 1979). Additional population reductions of codling moth can occur in sex pheromone-treated orchards with higher rates of mating disruption and greater delays of mating. For example, the proportion of females mated in sex pheromone-treated versus untreated walnut orchards was reduced 40% during the first and second moth flight periods (Light et al. 2001). Significant delays, 7–10 d, (45–55 DD, lower developmental threshold = 10°C) in the onset of egg hatch in early summer have been shown in apple and pear orchards treated with sex pheromone dispensers (unpublished data).

The disruption and delay in mating by male codling moths could also be important components of the effectiveness of sex pheromones in reducing population growth. Male moths are able to mate on successive days under laboratory conditions. The order of mating under these conditions did not influence female fecundity or egg fertility. In contrast, previous studies reported reductions in fecundity with multiple male matings, although these data were not analyzed statistically (Hagley 1974, Howell 1980a). Male age, however, was a significant factor affecting fecundity. Significant reductions observed in the fecundity of female codling moths mating with naïve, <24-h-old males had not previously been reported (Howell 1991). This result may have been caused by asynchronous sperm release from male testes as a result of their variable temporal exposure (1–24 h) to a reversed photoperiod before mating (Giebertowicz and Brooks 1998). The importance of this finding for field populations is unknown.

Moth age of experienced males (second and third mating event) was a significant factor reducing egg fertility by as much as 40%. While, the male moth's age

Table 4. Mean no. and density distribution of spermatophores in mated female codling moths caught in pear ester–baited traps placed in orchards treated with and without sex pheromone dispensers for mating disruption (MD), 2000–2003

Treatment ^a	No. moths	Density distribution of spermatophores in mated female moths						Mean (SE) no spermatophores
		1	2	3	4	5	6	
First moth flight								
No MD	1,668	0.950	0.046	0.002	0.001	0.001	0.0	1.06 (0.01)a
MD _{500–750}	1,435	0.981	0.016	0.003	0.0	0.0	0.0	1.02 (0.004)b
MD _{1,000}	1,448	0.977	0.020	0.003	0.0	0.0	0.0	1.03 (0.005)b
Kruskall-Wallis test: df = 2,4548								F = 14.8; P < 0.0001
Second moth flight								
No MD	1,483	0.690	0.212	0.073	0.017	0.005	0.003	1.44 (0.02)a
MD _{500–750}	780	0.874	0.121	0.003	0.002	0.0	0.0	1.14 (0.01)b
MD _{1,000}	1,305	0.924	0.066	0.005	0.005	0.0	0.0	1.08 (0.01)c
Kruskall-Wallis test: df = 2,3658								F = 170.0; P < 0.001

Column means within each moth flight followed by a different letter were significant different (LSD test, $P < 0.05$).
^a Orchards were treated with sex pheromone for mating disruption (MD) of codling moth. The subscript represents the no. of Isomate-C Plus dispensers applied per hectare.

when females were mated in the field is unknown, it is more likely that females with small spermatophores were mated by older males. For example, the mean (SE) age of males transferring a small (≤ 1.5 mm) versus a large (> 1.5 mm) spermatophore was 4.2 (0.2) versus 1.7 (0.1) d under laboratory conditions. Whether the significant increase in the proportion of females with small spermatophores in pheromone-treated orchards could account for significant reductions in egg fertility is an important but unanswered question.

Evaluating the potential impacts of delayed or sequential mating by older male codling moths in the field is difficult for several reasons. First, egg fertility has not been evaluated for field populations of codling moths because of the typical low density of eggs present in orchards. Second, the validity of extrapolating egg fertility data from various laboratory colonies to field populations is unknown. Third, relating moth age (d) under constant, high temperatures in the laboratory with either cooler (spring) or warmer (summer) fluctuating temperatures in the field is difficult. Physiological time (degree days) is likely a better scale to measure quantitative and qualitative changes in ovigenesis and spermatogenesis. Fourth, the occurrence and importance of male adult feeding in the field is unknown. Live-trapping of moths with pear ester-baited light traps may be a useful approach to examine these impacts in future studies.

The prior mating status of male codling moths that mated with trapped female moths was estimated based on spermatophore size. While type 1 and 2 errors associated with this bimodal classification were low (< 0.10), factors other than mating order, unfortunately, can impact spermatophore size. Interruption of mating caused by disturbances from other moths or from the environment has been suggested as factors contributing to small spermatophores (Howell 1991). For example, the transfer of a large spermatophore required at least 30 min, whereas subsequent mating events transferred only a small spermatophore in 45–75 min (Howell et al. 1978). Male–male mate competition has influenced both mating success and spermatophore size in laboratory tests with codling moth (Howell et al. 1978). Unfortunately, spermatophores were measured in this study only from female moths trapped in orchards with high moth densities where mate competition may occur. The presence of high levels of atmospheric sex pheromone could also conceivably affect certain short-range courtship behaviors in codling moth that may impact spermatophore size. Exposure of virgin females to sex pheromone-laden air significantly increased their level of sexual activities, such as calling (Weissling and Knight 1996), but the duration or success of mating under these conditions have not been examined in the laboratory or contrasted in orchards treated with or without sex pheromone.

Table 5. Comparison of spermatophore sizes in single and multiply mated 3 female codling moth from conventional and sex pheromone-treated (MD) orchards, 2003

Treatment	Flight	Mean (SE) length of spermatophore			Proportion of spermatophores ≤ 1.5 mm	
		Singly mated	Multiply mated	Unpaired <i>t</i> -test	Singly mated	Multiply mated
Conventional	First	1.66 (0.02) aA	1.54 (0.06) B	$t_{289} = 3.27$; $P < 0.01$	0.19b	0.31
MD-treated	First	1.58 (0.03) bA	1.33 (0.08) B	$t_{79} = 2.34$; $P < 0.05$	0.30a	0.50
ANOVA: $F_{1,321} = 4.03$; $P < 0.05$				$F_{1,51} = 3.89$; $P = 0.05$	$\chi^2 = 3.87$; $P < 0.05$	$\chi^2 = 2.57$; $P = 0.05$
Conventional	Second	1.55 (0.07) a	1.54 (0.06)	$t_{83} = 0.16$; $P = 0.88$	0.38b	0.38
MD-treated	Second	1.25 (0.10) b	1.33 (0.10)	$t_{28} = 0.75$; $P = 0.46$	0.75a	0.57
ANOVA: $F_{1,48} = 6.17$; $P < 0.05$				$F_{1,49} = 3.43$; $P = 0.07$	$\chi^2 = 6.17$; $P < 0.05$	$\chi^2 = 2.15$; $P = 0.14$

Column means of spermatophore length followed by a different lowercase (ANOVA) and row means followed by a different capital letter (unpaired *t*-test) are significantly different ($P < 0.05$). Column means for the proportion of small spermatophores in each flight followed by a different letter are significantly different (χ^2 2 by 2 contingency test, $P < 0.05$).

Understanding why nearly 70% of mated females in sex pheromone-treated orchards possessed a single, small spermatophore is important in assessing the effectiveness of sex pheromones for codling moth. These data suggest that experienced males were much more effective than naïve males in mate acquisition in sex pheromone-treated versus untreated orchards. Thus, effective disruption of mating for codling moth with sex pheromone may be difficult because of successful mate acquisition by only a small proportion of the male population. Other factors could conceivably affect the success of "experienced" males. Duthie et al. (2003) suggested that the recently identified larval aggregation pheromone and the precocious release of pheromone by female pupae could increase mating success of codling moth within clumped patches. In addition, visual cues are known to be important in mate acquisition (Castrovillo and Carde 1980) and would be enhanced by a high-density clumped distribution of moths. Several studies with other tortricid species have reported that pre-exposed males are more responsive (longer upwind flights) to a sex pheromone source than naïve males (Sanders 1985; Stelinski et al. 2004, 2005). In these studies, both the blend and pheromone rate were important factors affecting postexposure behaviors. Male codling moth antennal receptors respond to nine compounds found in the female effluvia (Witzgall et al. 2001), and laboratory studies have suggested that a multicomponent blend is more attractive than codlemone alone (El-Sayed et al. 1999, Witzgall et al. 2001). Future studies should examine the effect on male behaviors after pre-exposure to various blends of these compounds at both female-equivalent and dispenser emission rates.

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